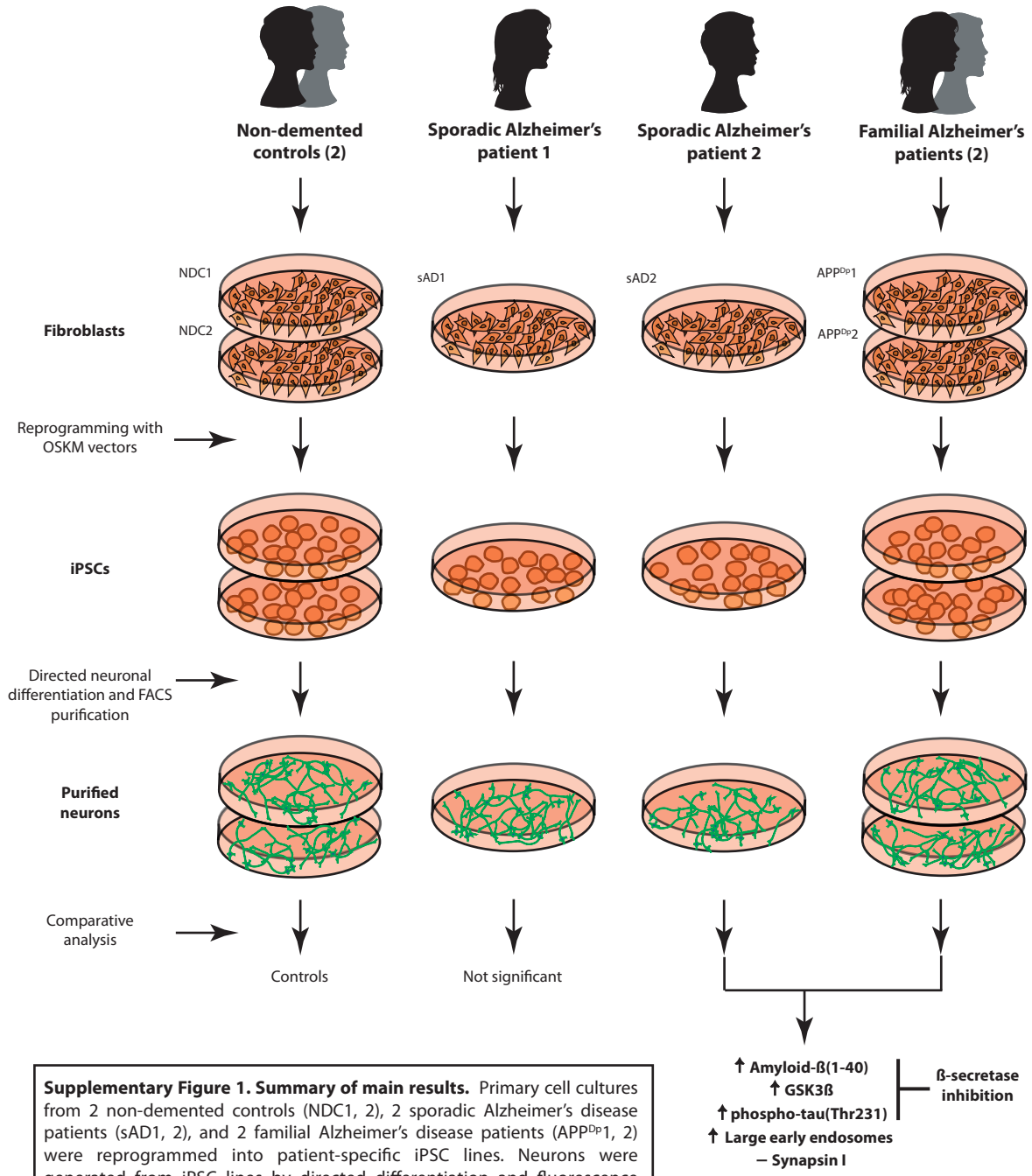
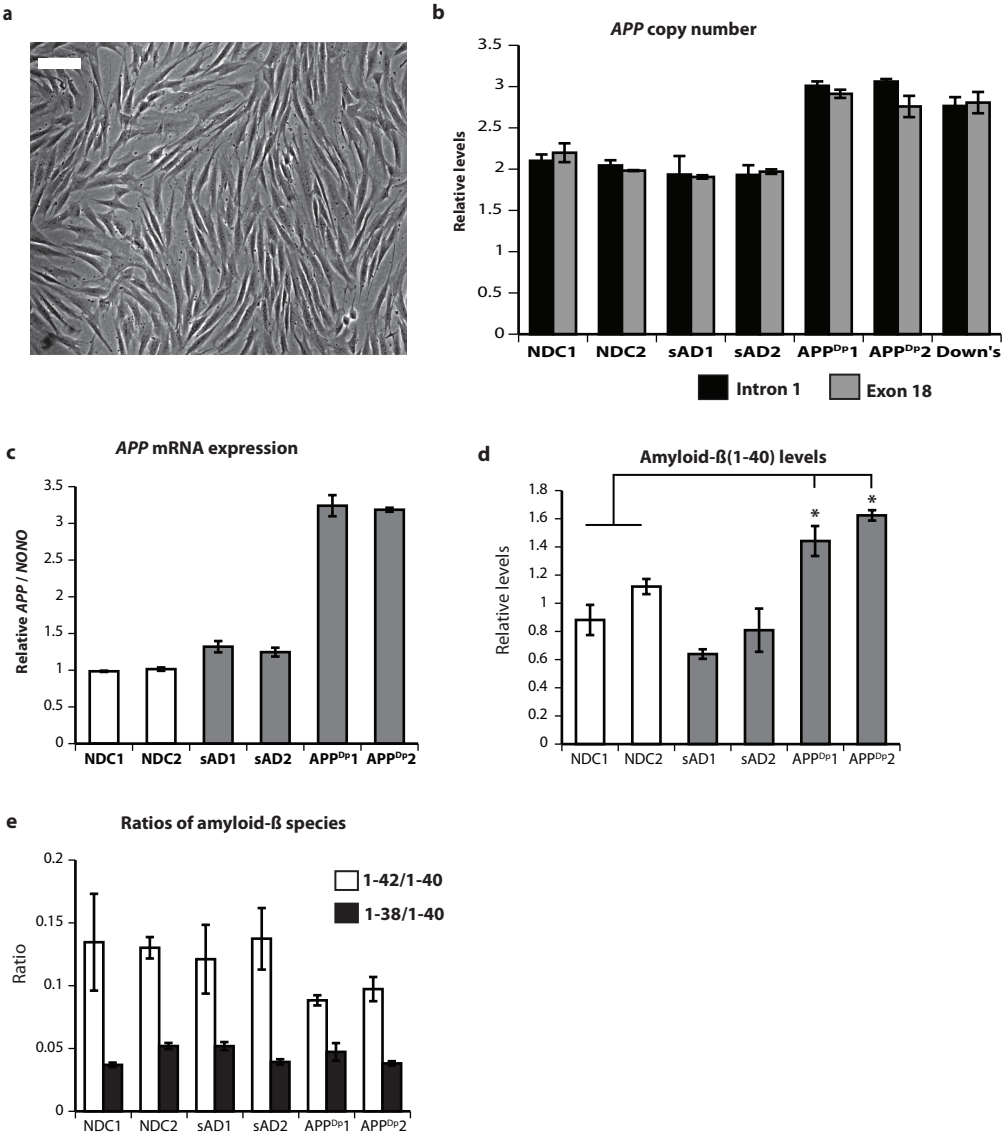
SUPPLEMENTARY INFORMATION

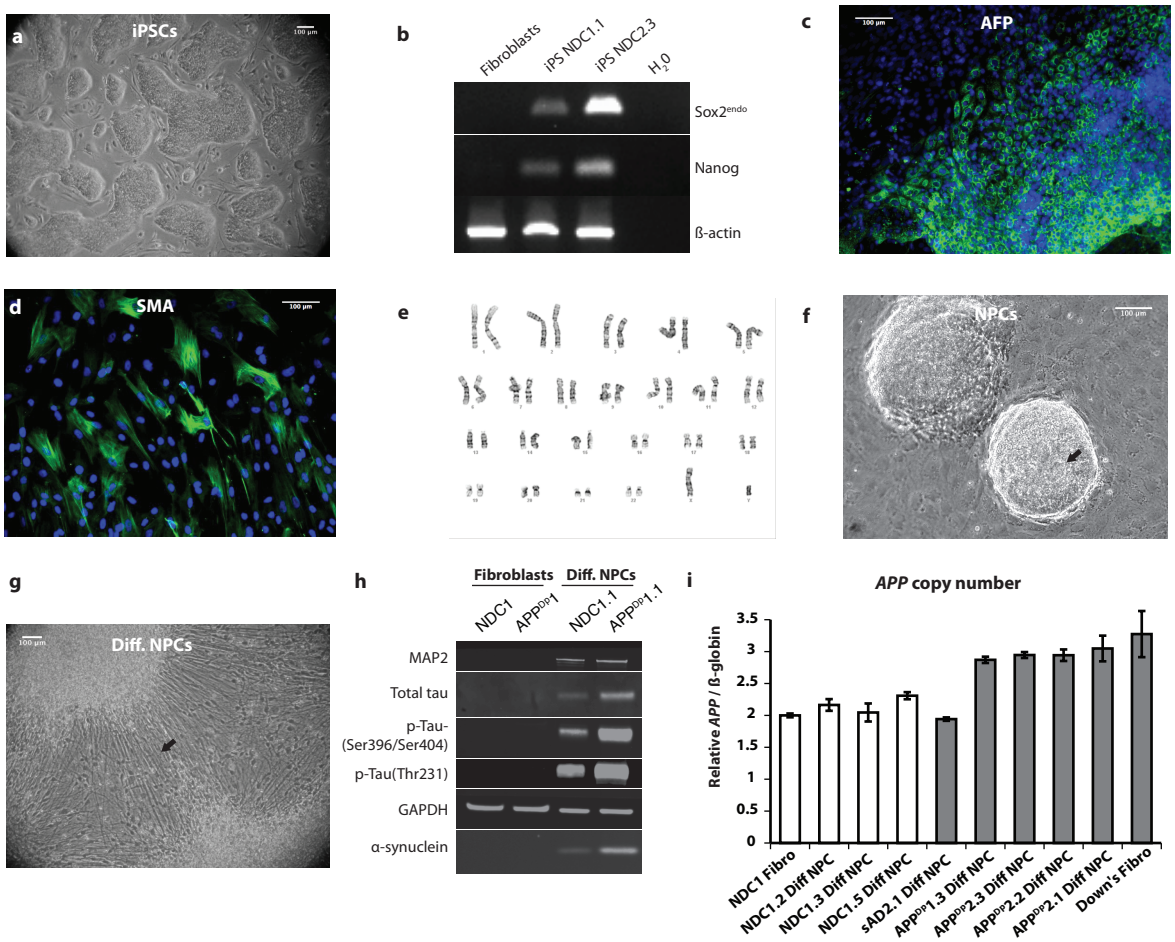
doi:10.1038/nature10821



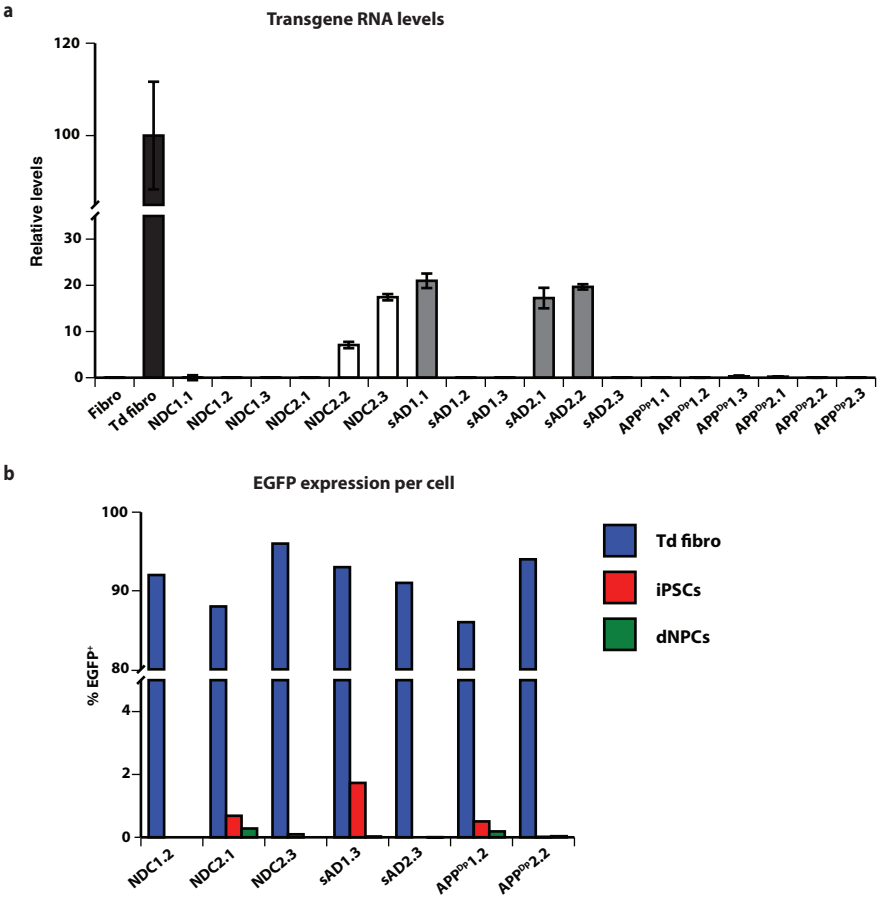
Supplementary Figure 1. Summary of main results. Primary cell cultures from 2 non-demented controls (NDC1, 2), 2 sporadic Alzheimer's disease patients (sAD1, 2), and 2 familial Alzheimer's disease patients (APP^{Dp}1, 2) were reprogrammed into patient-specific iPSC lines. Neurons were generated from iPSC lines by directed differentiation and fluorescence activated cell sorting (FACS) purification. Purified neurons from sAD2 and APP^{Dp} had significantly higher levels of secreted Aß(1-40), active GSK3ß, phospho-tau, and large $RAB5^{+}$ endosomes relative to NDC neurons. Pharmacologic inhibition of ß-secretase caused a significant reduction in the levels of Aß(1-40), phospho-tau and active GSK3ß.



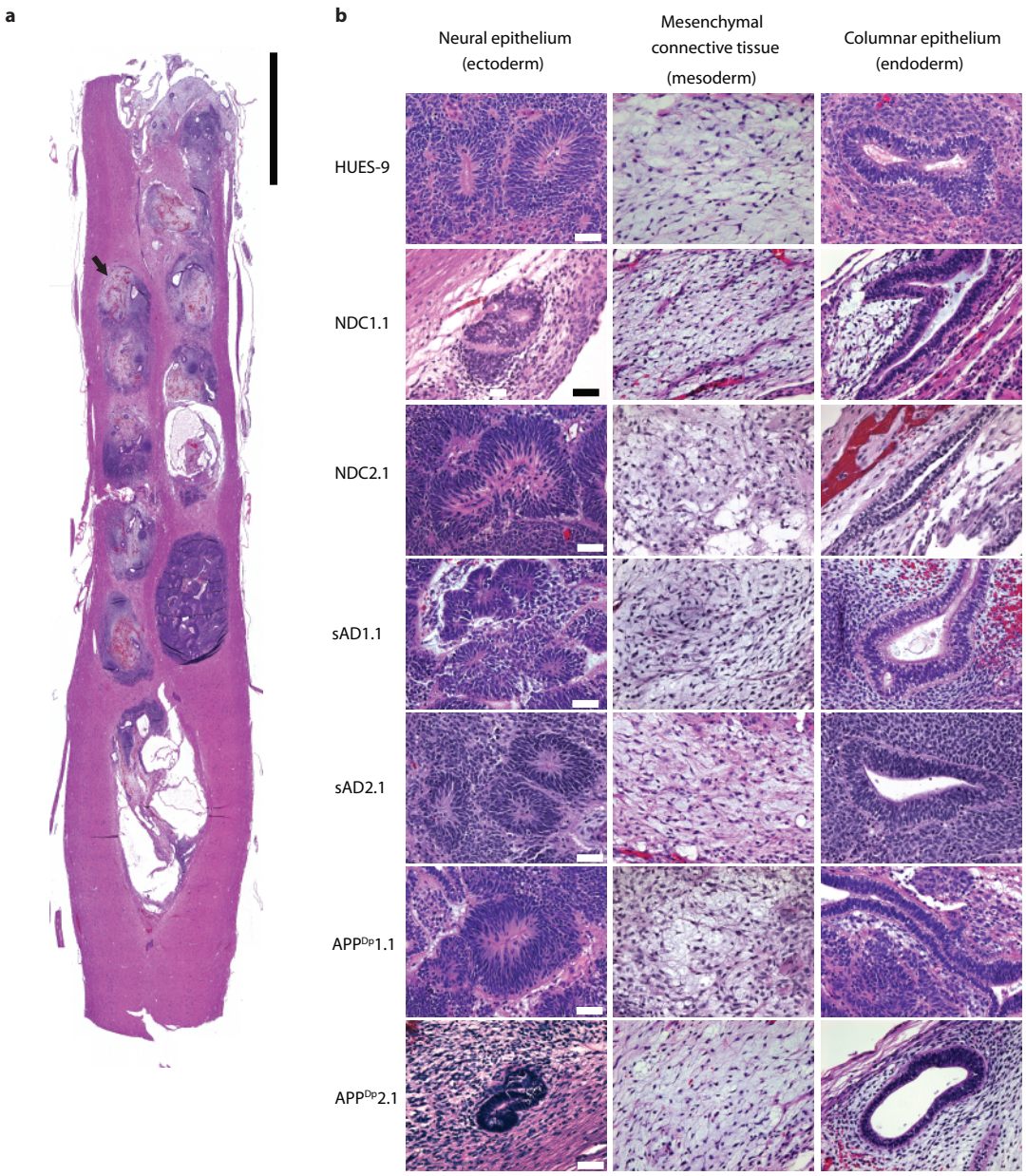
Supplementary Figure 2. Additional characterization of patient fibroblasts. a, Representative brightfield image of fibroblast cultures (line NDC1 shown). Scale bar marks 100 µm. **b**, Familial Alzheimer's disease samples contained 3 copies of the *APP* locus, while other samples were diploid. Down's, Down's syndrome fibroblasts. Quantitative PCR for two regions of the *APP* gene was performed on genomic DNA preparations and normalized to β-globin levels. **c**, Familial Alzheimer's disease fibroblasts expressed higher levels of *APP* mRNA relative to NDC and sAD samples. *APP* expression levels were normalized to expression levels of the housekeeping gene *NONO*. **d,** $APP^{Dp}1$ and 2 fibroblasts secrete increased levels of amyloid-ß(1-40) compared to NDC cells ($P = 0.01$ and 0.0008, respectively, $n = 3$). **e,** No significant difference in amyloid-ß- (1-42/1-40) or (1-38/1-40) between patients.



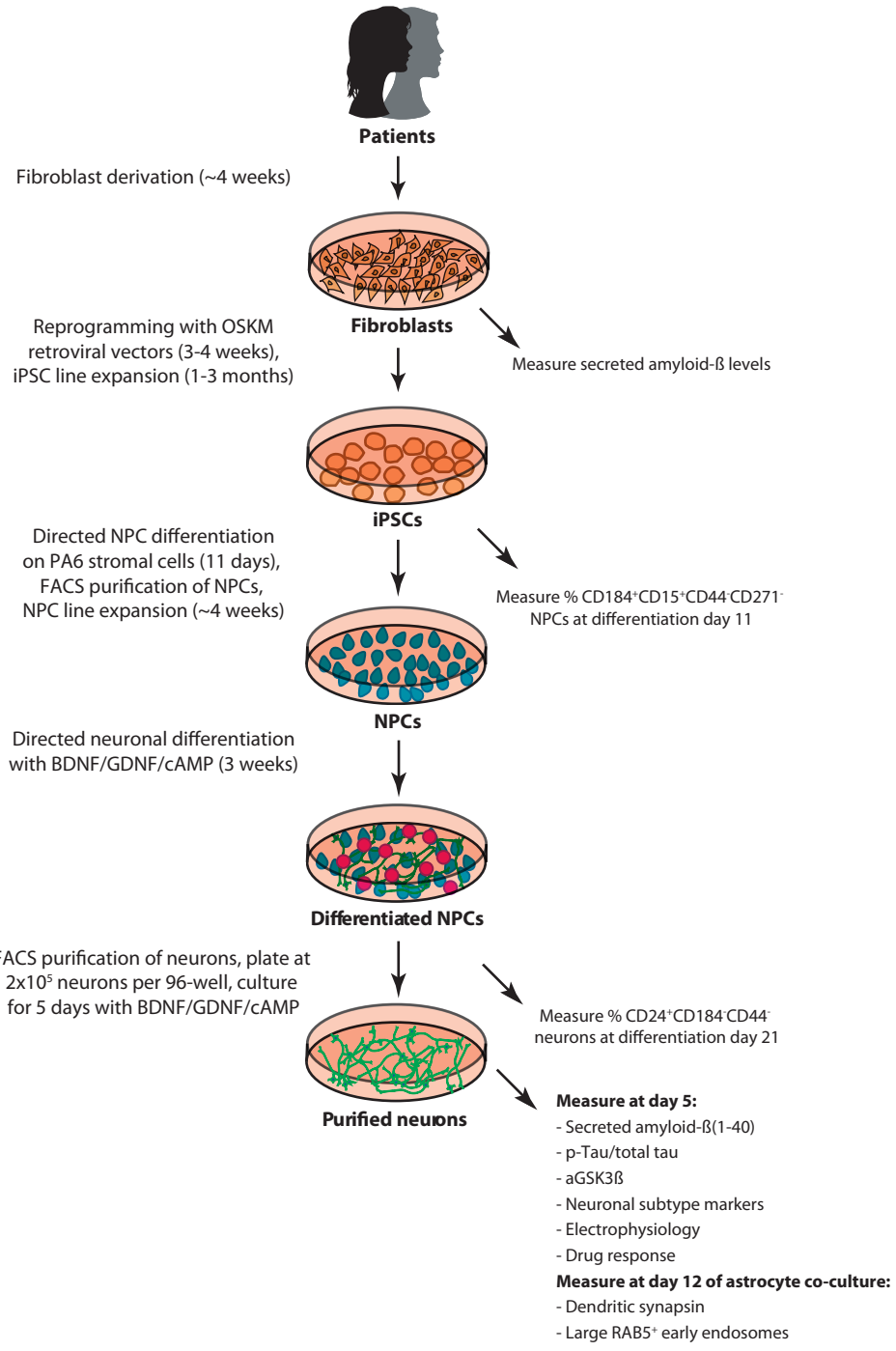
Supplementary Figure 3. Additional characterization of iPSC lines. a, Representative brightfield image of an iPSC line co-cultured on MEFs (Line NDC1.2 shown). Note hESC-like morphology. **b-e**, All iPSC lines expressed SOX2 derived from the endogenous locus, formed embryoid bodies that contained cells indicative of endodermal and mesodermal germ layers, and maintained euploid karyotypes (representative data shown). AFP, α-fetoprotein (endodermal); SMA, α-smooth muscle actin (mesodermal). **f**, Representative brightfield image of an iPSC line differentiated on PA6 stromal cells for 11 days. Arrow indicates neural rosette-like structure. **g**, Brightfield image of NPCs differentiated for 3 weeks. Arrow indicates neuronal projections. Note difference in homogeneity with FACS purified neurons (Figure 1h). **h**, Neuronal markers and phosphorylated tau (p-tau) were detected in cultures of NPCs differentiated for 3 weeks. Note the absence of tau in fibroblasts. **i**, *APP* copy number was correctly maintained in iPSC-derived NPCs differentiated for 3 weeks. Quantitative PCR specific to the *APP* gene was performed on genomic DNA preparations and normalized to β-globin levels.



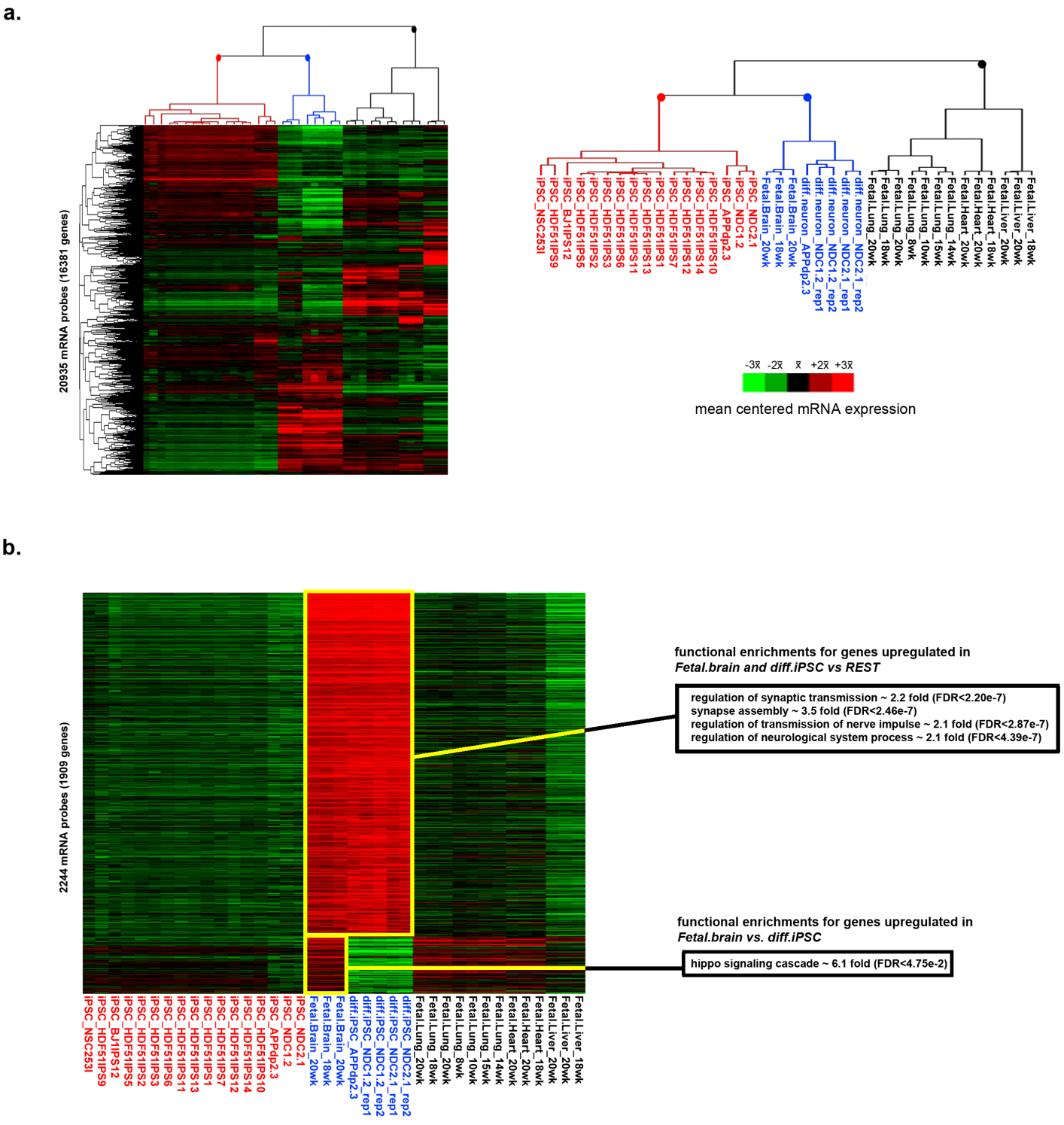
Supplementary Figure 4. Retroviral transgene repression in iPSCs. a, Transgene RNA expression levels in undifferentiated iPSCs relative to fibroblasts 3 days after retroviral transduction ("Td fibro"). Primers detected a tag common to all transgenes. Expression levels were normalized to levels of the housekeeping gene *NONO*. **b**, The percentage of EGFP$^+$ cells in iPSCs, differentiated NPCs (dNPCs) and their parental transduced fibroblasts.



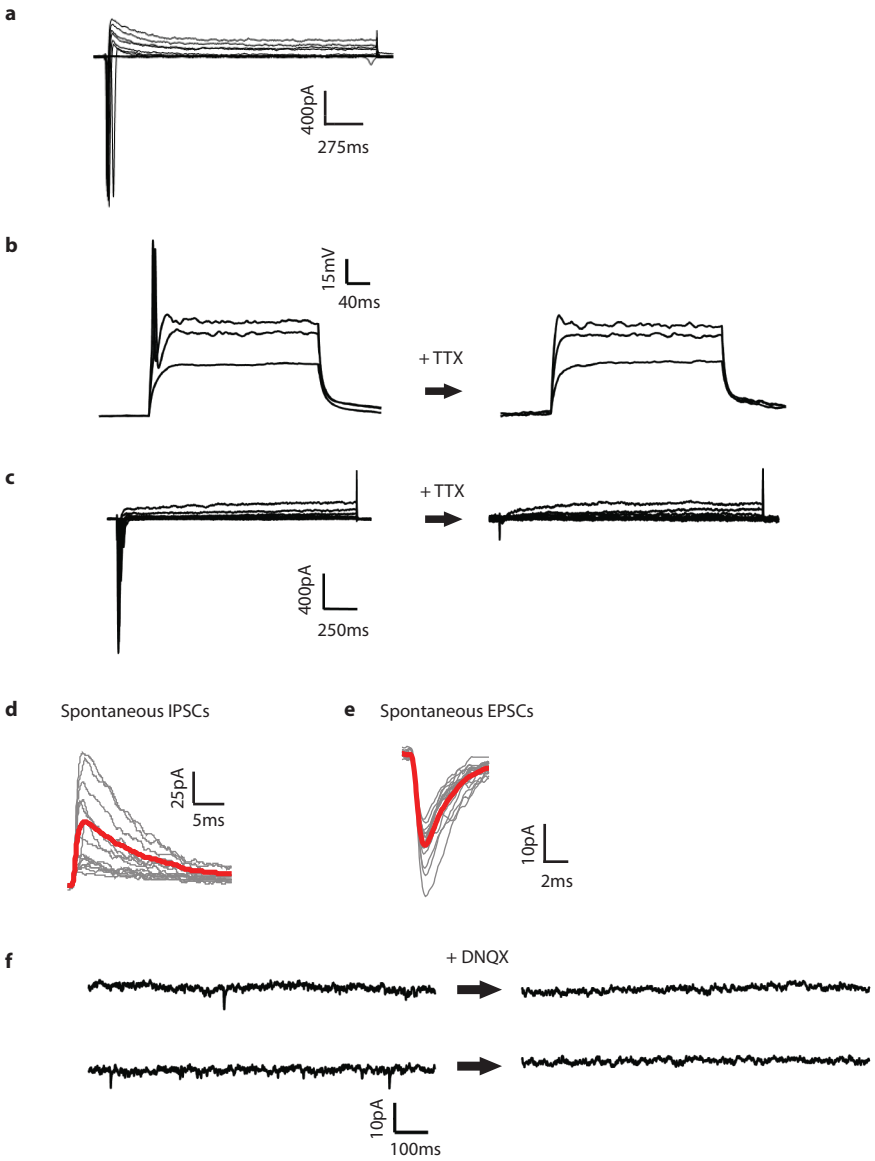
Supplementary Figure 5. Teratoma formation. One iPSC line per individual plus an ESC line (HUES-9) was tested for pluripotency *in vivo* by ten bilateral injections into lumbar spinal cords of nude rats (see methods). **a**, H&E stained horizontal section of a whole spinal cord showing the formation of multiple teratomas in individual injection sites per animal (line sAD1.1 shown). Arrow marks one injection site. Scale bar, 2 mm. **b**, Higher magnification images showing the presence of ectodermal, mesodermal and endodermal lineages for each iPSC line. Scale bars, 50 µm.



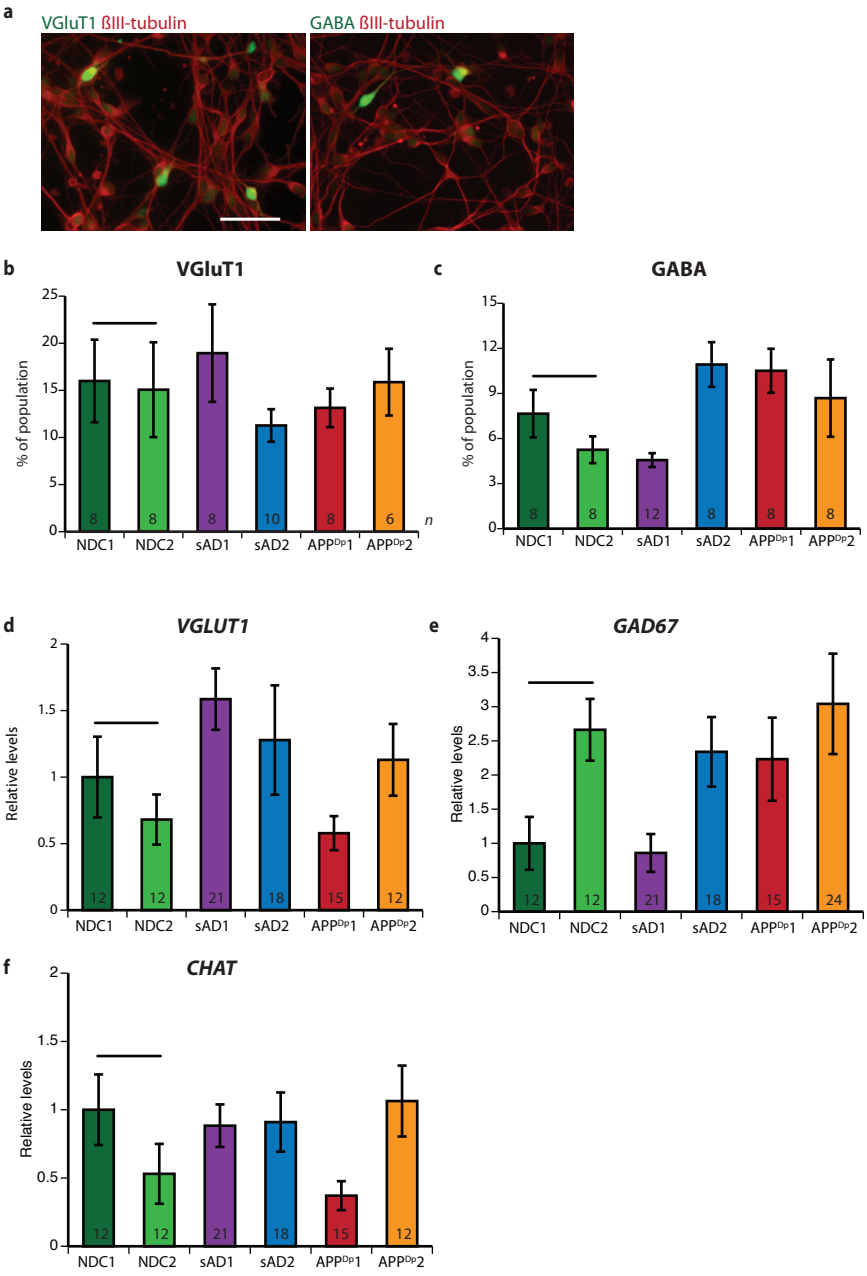
Supplementary Figure 6. Summary of differentiation method.



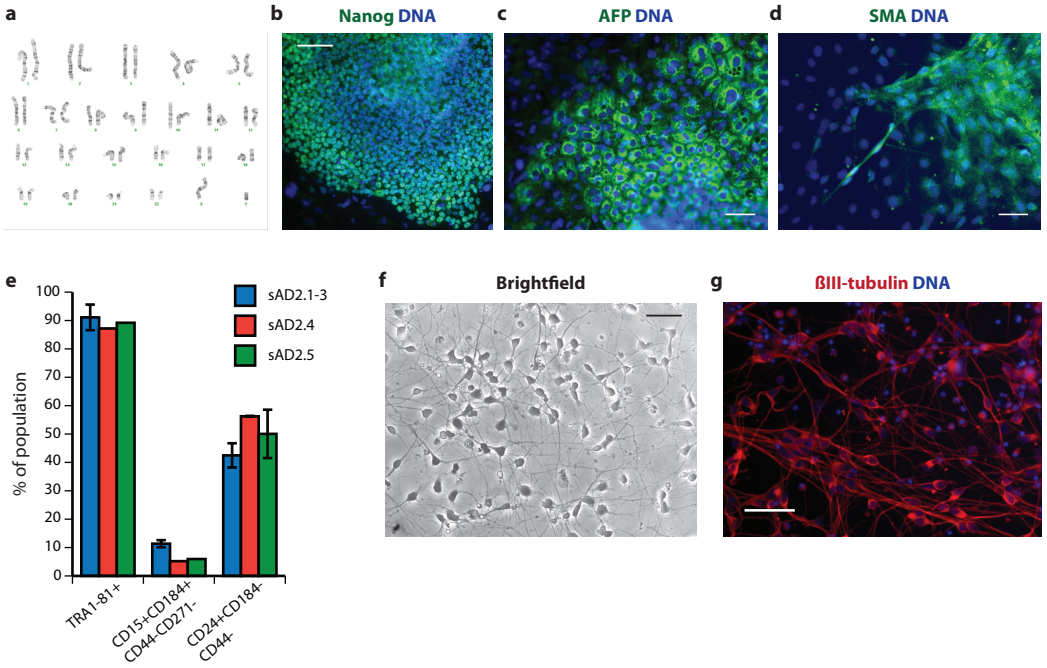
Supplementary Figure 7. Genome-wide mRNA expression profiling of purified *in vitro* differentiated neurons reveals global upregulation of neuronal genes and clustering with fetal brain samples. a, Unsupervised hierarchical clustering analysis of purified differentiated neurons, parental iPSCs, previously published iPSCs and fetal tissues, according to all probes with significant detection on the Illumina HT-12v4Expression BeadChip. Samples are clustered via Euclidean distance, and genes via Correlation metric using Cluster 3.0 (Eisen lab). iPSC_NDC1.2, iPSC_NDC2.1 and iPSC_APPdp2.3 are undifferentiated iPSCs from current study. Diff.neuron are purified differentiated neurons from current study. **b,** Heatmap of genes upregulated in fetal brain and differentiated neurons compared to all other samples, and genes up regulated in fetal brain versus differentiated neurons. Highlighted boxes signify upregulated gene groups and associated functional enrichments using G.R.E.A.T. Supplementary table 2 lists all differentially expressed genes (Fold change > 2.0, $P \leq 0.01$).



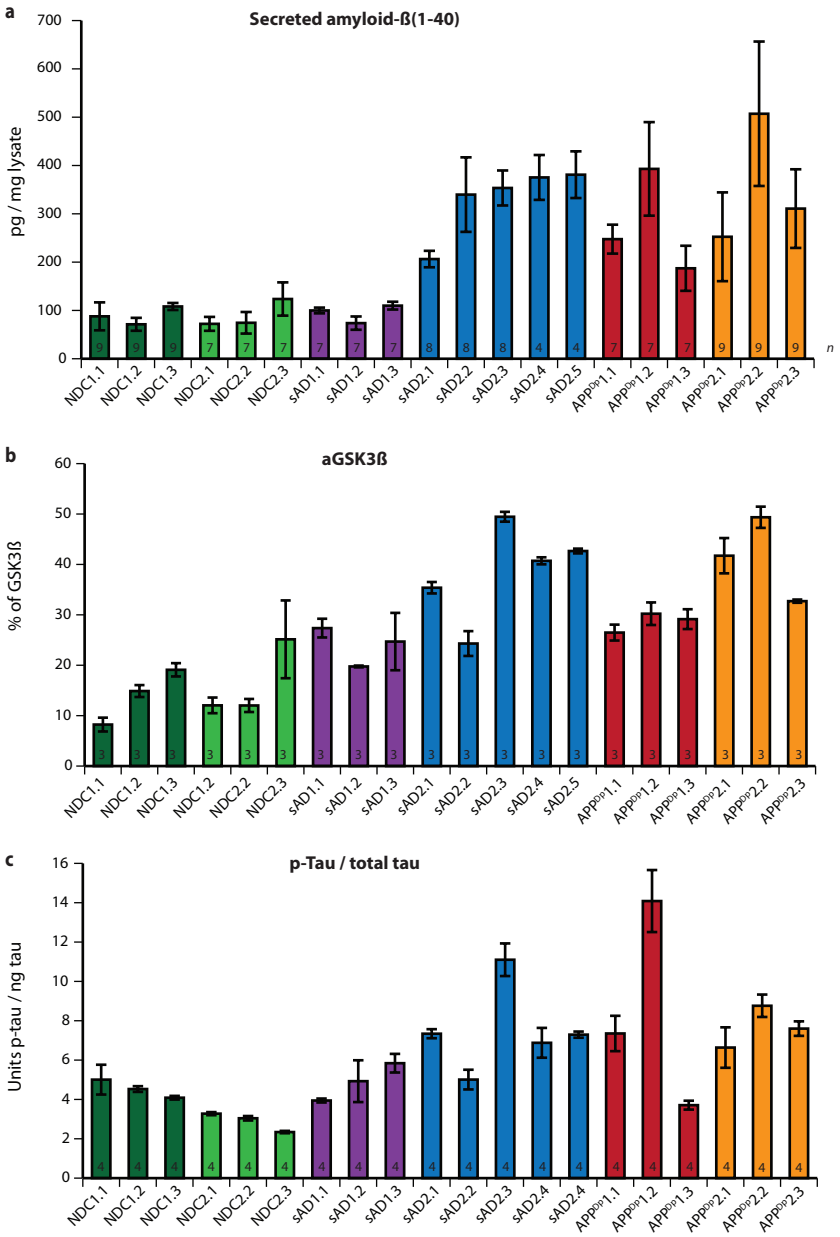
Supplementary Figure 8. Additional electrophysiological properties of purified neurons. a, 30 of 32 purified neurons recorded in voltage clamp exhibited voltage dependent sodium and potassium currents. **b, c,** Actions potentials and currents were blocked by the voltage-gated fast sodium channel blocker tetrodotoxin (TTX). **d, e,** To determine if the purified neurons were able to form functional synaptic contacts, we measured spontaneous current activity in voltage clamp. Typical spontaneous events which were detected in a 4 min recordings are superimposed (gray) and averaged (red). We found spontaneous currents at reversal potential of Na+ (0 mV), typical of GABAergic synapses (d). We also found spontaneous currents at the reversal potential of Cl- (-70 mV), typical of glutamatergic AMPA synapses (e). **f,** Treatment with the AMPA receptor antagonist DNQX blocked spontaneous EPSCs. GABAa receptor antagonist blocked spontaneous IPSCs (see Fig. 1j). Experiments were performed on samples from iPSC lines NDC2.3, sAD2.1 and APPDp2.2. See also summary of findings in Supplementary Table 3.



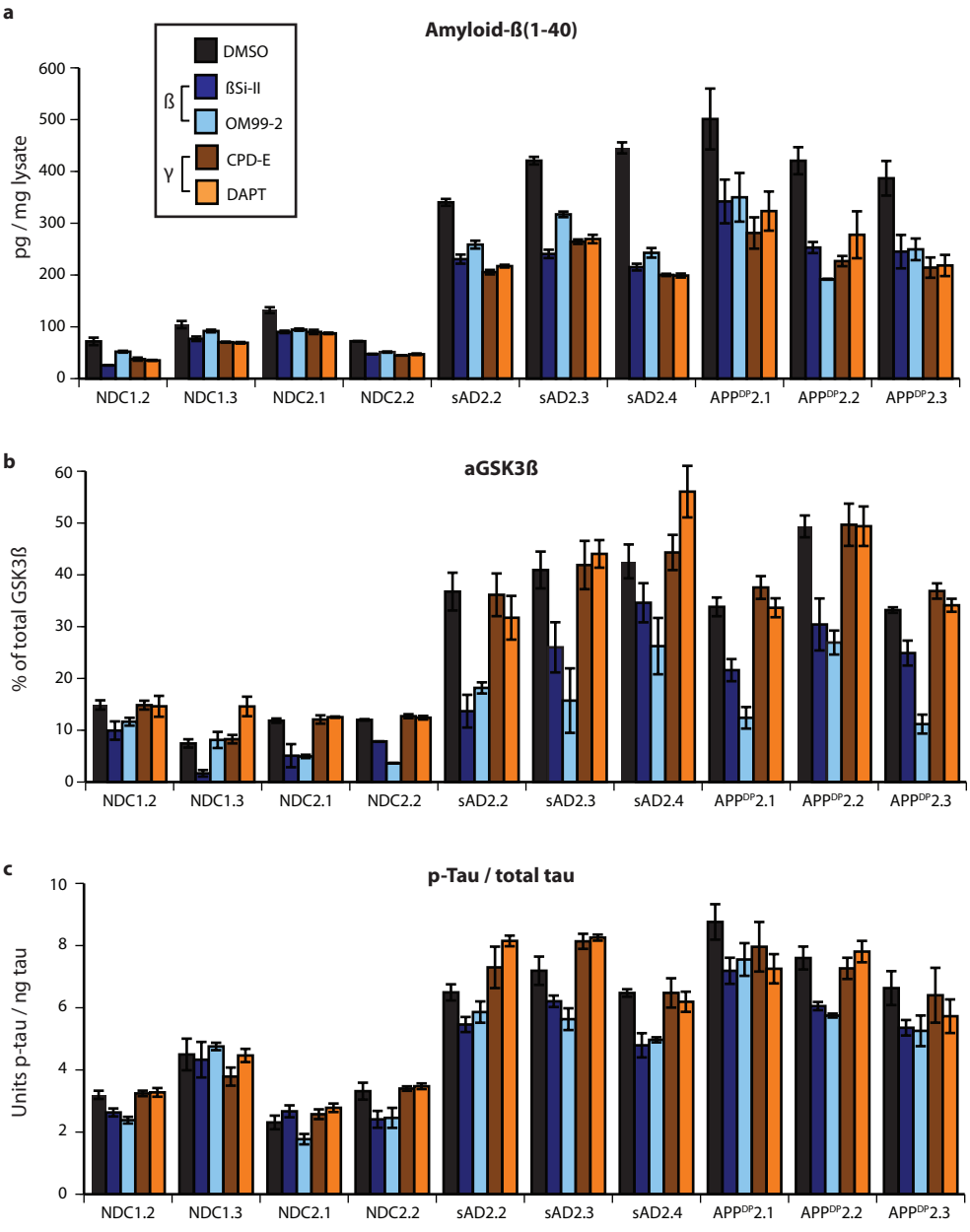
Supplementary Figure 9. No significant difference in neuronal subtype expression between patients and controls. a, Representative immunofluorescent (I.F.) images showing expression of VGluT1 and GABA (glutamatergic and GABAergic markers, respectively) in purified neuronal cultures. Scale bars mark 50 µm. **b,c**, Quantification of I.F. results. No significant difference was observed in the percentage of VGluT1^{+} or GABA^{+} cells between patients and controls (VGluT1 $P = 0.62$ and GABA $P > 0.12$). Each individual was represented equally by 2 iPSC lines. For GABA, sAD1 was significantly different than sAD2 and APP^{Dp}1 ($P = 0.02$ and 0.03). **d-f**, Quantification of neuronal subtype RNAs. QPCR was performed on purified neurons with primers specific to *VGLUT1*, *GAD67*, and *CHAT* (glutamatergic, GABAergic, and cholinergic markers, respectively) and normalized to levels of the average of two housekeeping genes (*TBP* and *NONO*). Each individual is represented equally by 3 iPSC lines. None of the patient samples were significantly different than NDC samples. QPCR and I.F. samples were from separate differentiations.



Supplementary Figure 10. Characterization of iPSC lines sAD2.4 and sAD2.5. Both iPSC lines, **a**, maintained euploid male karyotypes, **b**, expressed nanog (scale bar, 100 µm), **c-d**, formed embryoid bodies that contained cells indicative of endodermal and mesodermal germ layers (scale bars, 50 µm). **e,** Compared to the initial sAD2 iPSC lines, sAD2.4 and sAD2.5 both possessed similar percentages of TRA1-81+ cells, and differentiated at similar efficiences from iPSCs to NPCs and from NPCs to neurons. *N* = 100,000 events. **f**, **g**, Representative images of FACS-purified neurons (line sAD2.4 shown, scale bars, 50 µm).



Supplementary Figure 11. Additional biochemical analysis of purified neurons. Levels of amyloid-ß(1-40), aGSK3ß, and p-tau/total tau of each iPSC line.



Supplementary Figure 12. Effect of ß- and γ-secretase inhibitors per iPSC line.

Table S1. Summary of iPSC lines.

iPSC line	Patient	Vectors used*	Trans-duction well number	Fibroblast passage number at transduct-ion	Mean % TRA1-81^{+}†	Transgene expression ‡	iPSC passage number at differentia-tion	iPSC passage number at karyotype	Mean % NPC§	Mean % neuron¶
NDC1.1	NDC1	OSKM	1	5	82.3	<0.001	7	8	8.4	54.5
NDC1.2	NDC1	OSKMG	2	5	91.0	<0.001	6	8	8.7	35.3
NDC1.3	NDC1	OSKM	3	5	84.7	<0.001	10	10	5.3	38.5
NDC2.1	NDC2	OSKMG	1	5	81.9	<0.001	6	9	12.8	27.8
NDC2.2	NDC2	OSKMG	2	5	87.7	7.1	7	10	11.2	41.9
NDC2.3	NDC2	OSKMG	1	5	84.4	17.4	8	12	8.5	33.7
sAD1.1	sAD1	OSKM	1	5	85.3	21.0	9	7	13.0	34.8
sAD1.2	sAD1	OSKM	2	5	84.8	<0.001	7	7	5.4	41.1
sAD1.3	sAD1	OSKMG	3	5	85.8	<0.001	7	7	13.2	50.1
sAD2.1	sAD2	OSKM	1	5	94.8	17.2	9	14	11.2	35.2
sAD2.2	sAD2	OSKM	2	5	82.1	19.7	8	15	5.0	44.1
sAD2.3	sAD2	OSKMG	3	5	96.4	<0.001	4	11	9.4	48.1
sAD2.4	sAD2	OSKMG	3	5	87.2	<0.001	10	11	5.2	50.1
sAD2.5	sAD2	OSKMG	3	5	89.2	<0.001	10	15	6.0	56.3
APP^{Dp}1.1	APP^{Dp}1	OSKM	1	5	81.0	<0.001	9	9	3.8	26.5
APP^{Dp}1.2	APP^{Dp}1	OSKMG	2	5	81.8	<0.001	7	11	5.8	49.3
APP^{Dp}1.3	APP^{Dp}1	OSKM	3	5	83.8	0.3	6	9	9.9	35.1
APP^{Dp}2.1	APP^{Dp}2	OSKM	1	5	91.4	0.2	11	11	7.4	43.7
APP^{Dp}2.2	APP^{Dp}2	OSKM	2	5	76.7	<0.001	6	7	15.2	33.5
APP^{Dp}2.3	APP^{Dp}2	OSKMG	3	5	86.7	<0.001	6	6	10.0	45.5

***** OSKM (OCT4, SOX2, KLF4, cMYC), OSKMG (OSKM + EGFP)
† Including MEFs, $n > 20{,}000$
‡ Percentage relative to transduced fibroblasts
§ After iPSC differentiated on PA6 for 11 days, before FACS purification, $n > 30{,}000$
¶ After NPCs (passage 7) differentiated for 3 weeks, before FACS purification, $n > 30{,}000$

Table S3. Summary of physiological properties of purified neurons.

Data recorded from iPSC lines NDC2.3, sAD2.1 and APP^{Dp}2.2.

Passive properties	**Mean**	**S.E.M.**	***n***
Input resistance	1497 MΩ	± 156	15
Capacitance	14 pF	± 0.79	15
Action potential properties	**Mean**	**S.E.M.**	***n***
Number of cells with action potentials	-	-	30 of 32
Amplitude from firing threshold	43.3 mV	± 7.5	7
Peak of voltage dependent sodium channel current	-828 pA	± 160	30
Minimum threshold to spike	-37 mV	± 2	30
Threshold to evoke maximum sodium currents	-24 mV	± 2.6	30
sIPSC events	**Mean**	**S.E.M.**	***n***
Number of cells with sIPSCs	-	-	4 of 9
Frequency	0.13 Hz	± 0.10	4
Amplitude	24 pA	± 7	4
Rise time 10-90%	3 ms	± 1	4
Decay time 10-90%	42 ms	± 15	4
Bath application of GABA receptor agonist	**Mean**	**S.E.M.**	***n***
Number of cells responding to 25 μM Muscimol	-	-	6 of 8
Peak response	123 pA	± 25	6
Recovery after wash out	94 %	± 6	4
Current injected to hold cell at 0 mV	13 pA	± 3	9
sEPSC events	**Mean**	**S.E.M.**	***n***
Number of cells with sEPSCs	-	-	2 of 11
Frequency	0.04 Hz	N/A	2
Amplitude	-20 pA	N/A	2
Rise time 10-90%	<1 ms	N/A	2
Decay time 10-90%	<5 ms	N/A	2
Bath application of AMPA receptor agonist	**Mean**	**S.E.M.**	***n***
Number of cells responding to 10 μM AMPA	-	-	1 of 7
Peak response	-4 pA	N/A	1
Recovery after wash out	100 %	N/A	1
Current injected to hold cell at -70 mV	-15 pA	± 3	11

Table S4. Means (a) and Pearson correlation coefficients (b) observed in purified neurons.

a

	Amyloid-β-(1-40) (pg/mg)	aGSK3β (%)	p-Tau/ total tau (units/pg)	Total tau (pg/mL)	Total protein (µg/mL)	*VGLUT1* (QPCR/IF %)	*GABA* (QPCR/ IF %)	*CHAT* (QPCR)
NDC1 mean	89	14	4.5	3.5	108	1.0 / 16.0	1.0 / 7.7	1.0
s.e.m.	11	2	0.3	0.9	10	0.3 / 4.3	0.5 / 1.6	0.6
NDC2 mean	90	16	2.9	3.7	126	0.9 / 15.1	0.7 / 5.3	2.2
s.e.m.	15	3	0.1	0.4	6	0.4 / 5.0	0.4 / 0.9	0.8
sAD1 mean	95	24	4.9	6.2	130	2.0 / 18.9	0.8 / 4.6	1.0
s.e.m.	8	2	0.4	0.3	5	0.8 / 5.2	0.4 / 0.5	0.8
sAD2* mean	300	36	7.8	6.8	145	1.8 / 11.3	1.2 / 10.9	2.1
s.e.m.	31	4	0.8	1.4	9	0.7 / 1.7	0.6 / 1.5	0.4
APP^{Dp}1 mean	276	29	8.4	5.3	98	0.7 / 13.1	0.6 / 10.5	1.7
s.e.m.	40	1	1.4	1.9	8	0.3 / 2.0	0.2 / 1.5	1.1
APP^{Dp}2 mean	357	41	7.7	7.4	142	1.4 / 15.9	1.2 / 8.7	2.8
s.e.m.	66	3	0.5	1.7	9	0.2 / 3.5	0.7 / 2.6	0.7

*** iPSC lines sAD2.1, sAD2.2 and sAD2.3**

b

	Amyloid-β (1-40)	aGSK3β	p-Tau/ total tau	Total tau	Total protein
Amyloid-β(1-40) correlation (*R*)	**1**	**0.94**	**0.91**	**0.76**	**0.33**
aGSK3ß *R*	**0.94**	**1**	**0.83**	**0.93**	**0.57**
pTau/tTau *R*	**0.91**	**0.83**	**1**	**0.71**	**0.07**

Very strong correlations ($R > 0.9$) indicated in red, strong correlations ($0.9 > R > 0.7$) in green, moderate correlations ($0.7 > R > 0.5$) in blue, and weak or no correlation ($R < 0.5$) in black.